

Remarks

This is in response to the Official Action of January 15, 2002. The points raised therein are addressed below.

The lack of a Figure 8 pointed out by the Examiner is amended herein by deleting reference to Figure 8 in the "Brief Description of the Drawings" section, and by modifying the discussion of these figures in the Examples to read simply "data not shown".

Claims 42-55 stand rejected as indefinite under the second paragraph of 35 USC 112 for various reasons discussed below. The many helpful suggestions provided by the Examiner are acknowledged with appreciation. The claims have been rewritten herein as discussed below, and it is respectfully submitted that these rejections may now be withdrawn.

All dependent claims have been amended to begin with "The" rather than "A", consistent with current USPTO usage.

Where the phrases have been interpreted in the singular by the USPTO in the Official Action, the applicants concur in that interpretation herein. Since it is proper usage to introduce an inherent feature of a structure with the article "the", it is respectfully submitted that the present response constitutes a complete response to this rejection.

In claims 42 and 55, the phrase "'construct comprising an expression cassette'" has been rewritten as simply "construct", since the phrase "expression cassette" is not utilized elsewhere in the claims, and so that the transition phrase "comprising" occurs only once in the claims.

Claim 43 has been amended to insert the phrase "wherein said nucleic acid is", as suggested by the Examiner.

The "of of" duplication pointed out by the Examiner in claim 48 has been corrected herein.

"In claims 50 and 54, "said plant cells" and "said plant" has been corrected to provide proper antecedent basis.

Claims 42-47 and 50-54 stand rejected under the first paragraph of 35 USC 112. To simplify the issues, claim 42 has been amended to incorporate claim 49, and

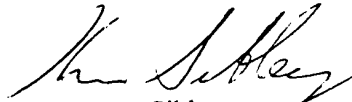
In re: Hanley-Bowdoin, et al.  
Serial No. 09/289,346  
Filed: 9 April 1999  
Page 6

claim 50 has been amended to incorporate claim 55. It is respectfully submitted that these amendments constitute a rewriting of claims 49 and 55 in independent form and not narrowing amendments thereof. Accordingly, it is respectfully submitted that this rejection has been rendered moot, and may now be withdrawn.

The changes made to the claims above are shown in the attached "**Version with Markings to Show Changes Made.**"

It is respectfully submitted that this application is in condition for allowance, which action is respectfully requested.

Respectfully submitted,

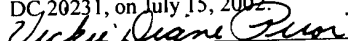


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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Box Non-Fee Amendment, Commissioner For Patents, Washington, DC 20231, on July 15, 2002.



Vickie Diane Prior

Date of Signature: July 15, 2002



**Version with Markings to Show Changes Made**

**In the Specification:**

Earlier studies have shown that TGMV AL1 and Rb interact with each other, but the region of the AL1 protein that mediates interaction was not known. The limits of the Rb binding domain were defined by the present inventors using a baculovirus expression system. Insect cells were co-infected with recombinant baculoviruses corresponding to various AL1 truncations and to a GST fusion with amino acids 214-866 of Maize Rb (GST-mRb). The abilities of the different AL1 truncation to bind GST-mRb were assessed by cofractionation on glutathione-sepharose resin. Total extracts and purified proteins were resolved by SDS-PAGE, and AL1 and GST-mRb were visualized by immunoblotting with AL1 and GST antibodies, respectively. The C-terminal truncation AL1(1-180) copurified with GST-mRb. Further deletion to amino acids 168 and 158 abolished interactions with GST-mRb. Similarly, the N-terminal truncation AL1(101-352) cofractionated with GST-mRb, whereas truncations at positions 110 and 119 were unable to bind GST-mRb. Together, these results mapped the limits of pRB binding domain between AL1 amino acids 101 and 180. Thus, the C-termini of the pRb binding and oligomerization domains of TGMV AL1 are contiguous, whereas an additional 33 N-terminal amino acids are required for Rb binding (data not shown). [ (see **Fig. 8A**).

**Figure 8B** shows protein interactions of C-terminal truncated AL1 proteins with GST-mRb. Input (lanes 1-3) and bound (lanes 4-6) fractions were resolved by SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting. AL1(1-180), lanes 1 and 4; AL1(1-168), lanes 2 and 5; AL1(1-158), lanes 3 and 6.

**Figure 8C** shows protein interactions of N-terminal truncated AL1 proteins with GST-mRb. Input (lanes 1-3) and bound (lanes 4-6) fractions were resolved by SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting. AL1(101-352), lanes 1 and 4; AL1(110-352), lanes 2 and 5; AL1(119-352), lanes 3 and 6. ]

**In the Claims:**

42 (amended). A nucleic acid construct [comprising an expression cassette], which construct comprises, in the 5' to 3' direction:

- (a) a promoter operable in a plant cell,
- (b) a nucleic acid sequence encoding a mutant AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and comprising a mutation in the Rb binding region, whereby binding of said mutant AL1 protein to a plant Rb protein is reduced compared to binding which would occur in the presence of a wild-type AL1 protein, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10; and
- (c) a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith.

43 (amended). The A nucleic acid construct according to claim 42, wherein said nucleic acid construct is carried by a plant transformation vector.

44 (amended). The [A] nucleic acid construct according to claim 42, where said nucleic acid sequence encodes a trans-dominant negative mutant AL1 protein.

45 (amended). The [A] nucleic acid construct according to claim [42] 44, wherein said trans-dominant negative mutant AL1 protein has a mutation in a domain selected from the group consisting of the oligomerization domain, the DNA cleavage domain, and the ATPase domain.

46 (amended). The [A] nucleic acid construct according to claim 42, wherein said nucleic acid sequence encodes an AL1 protein with increased repression of transcription from the AL1 promoter, compared to a wild-type AL1 protein.

47 (amended). The [A] nucleic acid construct according to claim 42, wherein said promoter is constitutively active in said plant.

48 (amended). The [A] nucleic acid construct according to claim 42, wherein said nucleic acid sequence comprises a sequence selected from the group consisting [of] of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:8.

50 (amended). A nucleic acid construct [comprising an expression cassette], which construct comprises, in the 5' to 3' direction:

- (a) a promoter operable in [said plant cells] a plant cell,
- (b) a nucleic acid sequence encoding a mutant AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and comprising a mutation in the oligomerization domain to produce a trans-dominant negative mutant AL1 protein, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10; and
- (c) a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith.

51 (amended). The [A] DNA construct according to claim 50 carried by a plant transformation vector.

52 (amended). The [A] DNA construct according to claim 50, wherein said nucleic acid sequence further comprises a mutation in the Rb binding region, whereby binding of said mutant AL1 protein to a plant Rb protein is reduced compared to binding which would occur in the presence of a wild-type AL1 protein.

53 (amended). The [A] DNA construct according to claim 50, wherein said nucleic acid sequence encodes an AL1 protein with increased repression of transcription from the AL1 promoter, compared to a wild-type AL1 protein.

In re: Hanley-Bowdoin, et al.  
Serial No. 09/289,346  
Filed: 9 April 1999  
Page 10

54 (amended). The [A] DNA construct according to claim 50, wherein said promoter is constitutively active in a [said] plant cell.